Formulation and Evaluation of Niosomal gel of stigma and leaves of *Stigma maydis L.*and their Comparative Evaluation for hypolipidemic activity.

A synopsis submitted for the degree of Doctor of Philosophy

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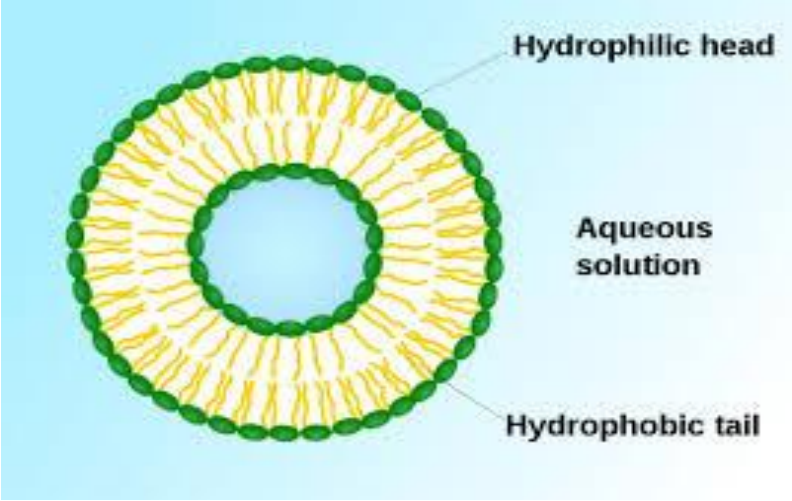
Introduction

Delivering drug with a controlled rate and targeted delivery received much attention in recent years .The application of nanotechnology to medicine has provide the development of nanoparticles used as drug carriers.

Nanocarriers present a great approach in drug delivery with promising features such as protection of drug from degradation and cleavage, controlled release and in case of targeted delivery approach the delivery of drug molecules to the target site.

Niosomes are one of the promising drug carriers that have a bilayer structure and are formed by self –association of non- ionic surfactants and cholesterol in an aqueous phase. Niosomes are biodegradable, biocompatible and immunogenic.



Components of Niosomes

1. **Surfactant**- Non-ionic surfactant are used as important structural components, they act as vesicles forming agents. The nature of vesicles formed depends upon HLB value in addition.

2. **Cholesterol**- Cholesterol acts vesicular cement in the molecular space that formed bilayer. There by increasing the rigidity decrease the permeability drug through the membrane and hence improves the entrapment efficiency.

3. **Solvents**- The solvents can acts as penetration enhancer and in turn affect the vesicular size formation ,solvents commonly used as alcohol, ethanol.

Methods of preparation

1. **Ether injection method**
   In this method surfactant mixture in ether is injected through 14-gauge needle into aqueous solution of material. Niosomes by slowing introducing a solution of surfactant dissolved in a diethyl ether into warm water maintained at 60°c.

2. **Thin film hydration technique**
   The mixture of vesicle forming ingredients like surfactant and cholesterol are dissolved in a volatile organic solvent (diethylether, chloroform or methanol) in a round bottom flask. The organic solvent is removed at room temperature using rotary evaporation leaving a thin layer of solid mixture deposited on the wall of the flask.

Stigma maydis L. is also known as corn hair. It is an essential herb that is used traditionally to treat a wide range of disease. It is a considered as a waste product of maize (corn).the male flowers called as tassels which produce yellow pollen. The female flowers produce corn silk. The silk functions as a stigma of a female flower, it is light brown colour. it contain bioactive components as flavanoids, terpenoids, polysaccrides.

Hyperlipidemic is a systemic metabolic disorder that is usually caused by un-healthy diet intake. It is characterised by abnormally elevated levels of total cholesterol, triglyceride, low density lipoprotein cholesterol (LDL-C), but decreased concentration of high density lipoprotein cholesterol in serum.

Hyperlipidemia is closely associated with many diseases such as cardiovascular disease, fatty liver and diabetes mellitus.

Currently used hypolipidemic drugs often trigger several adverse effects such as hyperuricemia, peripheral neuropathy, memory loss, muscle damage.

2. Aim and Objectives

The fundamental point of this study is to prepare and asses niosomes a hypolipidemic activity. The niosomes formulation will improve bioavailability and solubility and have also a sustained release profile.

To achieve the above mention aim following objectives

- To formulation of niosomes of *stigma maydis* extract.
- To conduct the different characterization of niosomes such as particle size,polydispersivity index and zeta potential
- To study morphology of the *stigma maydis* loaded niosomes by scanning electron microscopy (SEM) and transmission electron microscopy (TEM).
- To determine effects of cholesterol and non-ionic surfactant on, entrapment of *stigma maydis* extract and in vitro release of the niosomal vesicles.
- To study interaction between drug extract and components of niosomal gel using ATR-FTIR.
- To evaluation of physical state of extract in niosomal formulation through thermal analysis.
- To develop HPLC method for measuring average drug content of *stigma maydis* and validate it according to guidelines of ICH.
- To determine the stability of niosomal gel formulation on two distinctive temperatures 4-8°c and 25°c.
- To determine the *in-vitro* release of drug by employing dialysis methods
- To evaluate and compare the plasma level profiles of selected niosomal gel formulation.
- To determine and compare pharmacokinetics parameters of optimized formulation.

3. Review of Literature

1. Nessa et al studied the effect of gel formulation from corn silk (*stigma maydis*) in **healing burns** in white rats.

2. Wha Ae, Ha et al Reported therapeutic efficacy of corn silk extract by determined the acute and sub acute toxicity of corn silk extract in ICR mice.

3. George et al Investigated effect of corn silk aqueous extracts on intraocular pressure of systemic and non- systemic hypertensive subjects.

4. Guo Jian you et al studied the effects of corn silk on **glycaemic metabolism**. Alloxan and adrenalin induced hyperglycaemic mice were used in the study.

5. Wang-Qiang Guang et al studied **anti- inflammatory** effects of corn silk in a rat model of carrageen in-induced pleurisy.

6. Mohammad Ali et al studied **antioxidant properties** of ethanol-water extract from corn silk were estimated by different methods

7. Guo, Hao studied **pro-apoptotic** and **anti-proliferative effects** of corn silk extract on human colon cancer cell lines.

8. Kim, SoRa et al studied corn silk extract improves benign **prostatic hyperplasia** in experimental model.

9. Ling-Li Zheng et al studied ultrasound- assisted extraction of total flavanoids from corn silk and their **antioxidants activity**.

10. Choi, Sang Yoon studied inhibitory effect of corn silk on skin pigmentation .In this study , the inhibitory effect of corn silk on melanin production was evaluated .This study was performed to investigate the inhibitory effect of corn silk on melanin production in melan-a cells protein expression.

11. R A Laeliocattleya et al studied The characteristics of corn silk (Zea Mays L.) herbal drinks tea with vacuum drying method as antioxidant. The purpose of this study was to determine the effect of temperature and drying time.

12. W Zhao et al studied anti- diabetic effects of polysaccharides from corn silk on normal and **hyperglycaemia rats.**

13. OJ, BR Sharma et al studied effect of *maydis stigma* water extract on **adipogenesis** and blood glucose in **3T3-L1 adipocytes and db/db mice.**

14. Zyan et al studied microwave- assisted extraction and **antihyperlipidemic effects** of total flavonoids from corn silk.

15. H Nawaz et al studied effect of solvent polarity and extraction method on phytochemical composition and antioxidant potential of corn silk.

16. L Sheng et al studied **Anti- Diabetic potential** of corn silk in High- Fat Diet/Streptozotocin- induced Type 2 Diabetes Mice Model.

17. JA Udobang et al studied **antimalarial** and **antipyretic** activities of corn silk extract and fractions of *Zea Mays*.

18. Y Zhang et al studied **anti- diabetic**, **anti-oxidant** and **anti-hyperlipidemic** activities of flavonoids form corn silk on STZ-induced diabetic mice.

19. Pooyan Makvandi et al studied Biosynthesis and characterization of **antibacterial** thermo sensitive hydrogels based on corn silk extract, hyaluronic acid and nanosilver for potential wound healing.

20. Q Guo et al studied hypoglycaemic effects of polysaccharides from corn silk (*Maydis stigma*) and their beneficial roles via regulating the PI3K/Akt signalling pathway in L6 skeletal muscle.

21. L Alimin et al studied effects of corn silk (*Stigma Maydis*) Against Blood Glucose Levels of type 2 Diabetes Mellitus patients.

4. Research Envisaged

Niosomes are preferred over other vesicular system because of chemical stability, low toxicity. Niosomes which are non-ionic in nature has better availability of drug at the site of action and it has good intrinsic skin penetration.

There are many formulations available in the market to prevent hyperlipidemia but there is no topically niosomal gel containing stigma maydis for the treatment of hyperlipidemia.

Thus the aim of present research work will to prepare topically applied niosomal gel to increase the patient compliance.

The sustaining action of hyperlipidemia drug can be brought by entrapping the drug in the vesicular system of the non-ionic surfactant which will increase the drug in the blood circulation, hence help in the effective treatment of hyperlipidemia.

Stigma maydis contain polysaccharides reduce triglyceride levels through ATGL-(PPAR-α)/ (PGC-1α), (SREBP-1c)-ACC/FAS and ACC-CPT1 signal pathways.

Stigma Maydis also contain flavonoid, maysin. Maysin decrease the levels of intracellular lipid droplets and triglycerides (TG) and down regulated the protein expression levels of c/EBP, PPAR-Y AND ap2 in 3T3-L1 preadipocyte cells. Maysin inhibits lipid accumulation and adipocyte differention .it induces the apoptotic cell death in 3T3-L1 preadipocyte cells and activation of cascades which may lead to reduction of adipose tissue mass.

5. Hypothesis

- This proposed study evaluates the herbal formulation in the treatment of hyperlipidemia.
- Formulation of niosomes containing *stigma maydis* extract by modified ether injection method.
- Evaluation of niosomes for entrapment efficiency, drug content, means vesicle diameter and shape.
- Characterization of niosomes by **UV and FTIR**.
- To study *in vitro* release of drug from prepared Niosomes using modified diffusion cell apparatus.
- Interpretation of dissolution data by software.
- In this study- a water soluble stigma maydis **polysaccharide (SMP-1)** will isolate by the hot water extracting- alcohol precipitation method.
- SMP-1 will characterized by a series of analyses including high-performance gel permeation chromatography (HPGPC).
- SMP-1 will evaluate via testing bile acid-binding capacity in vitro and serum lipid- lowering effects in vivo. Collectively, this study will provide a solid evidence for the antihyperlipidemic effects of stigma maydis polysaccharide, thus offering a safe and effective functional food supplement for the treatment of hyperlipidemia and other associated chronic disease.

6. Work plan and Methodology

- Collection of plant material.
- Authentification of plant material by botanist.
- Pharmacognostic investigation of the plants.

6.1 **Preformulation studies**-

- Determination of solubility of drug.
- Determination of partition coefficient of drug.
- Melting point determination
- UV Spectral analysis
- IR Spectral analysis

6.2 **Drug Interaction Studies**-

- IR Spectroscopy

6.3 **Compatibility Studies between drug and cholesterol**

- IR Spectroscopy

6.4 **Preparation of standard curves of drug**-

- In phosphate buffer (Ph 7.4)
- In ethanol

6.5 **Preparation of niosomes**

6.6 **Characterization of niosomes-**

- Entrapment efficiency
- Particle size and shape
- Drug content
- *In vitro* release

6.7 **Optimization of formulation**

6.8 **Formulation of Niosomal gel**

6.9 **Characterization of prepare niosomal gel**

- Percentage yield
- Drug spreadbility
- Determination of pH of gel
- In vitro release
- In vivo release.

6.10 **Methods**

Analytical methods used for estimation of drugs either in bulk, niosomal gel or in dissolution media.

(a) Preparation of stock solution

To prepare solution 10 mg of the drug is dissolve in 10 ml ethanol individually, which is diluted further to give the final concentration ug/ml.

(b) Analytical methods

The UV Spectrophotometric methods are developing for estimation of drugs using UV software and IR spectrophotometer is utilized for structure.

Melting Point Determination

Melting point of drug sample will determine by capillary method using melting point apparatus.

Solubility Studies

The solubility studies will determine in different solvent.

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